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Microbiological control in stem cell banks: approaches to standardisation

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Abstract The transplant of cells of human origin is an increasingly complex sector of medicine which entails great opportunities for the treatment of a range of diseases. Stem cell banks should assure the quality, traceability and safety of cultures for transplantation and must implement an effective programme to prevent contamination of the final product. In donors, the presence of infectious micro-organisms, like human immunodeficiency virus, hepatitis B virus, hepatitis C virus and human T cell lymphotropic virus, should be evaluated in addition to the possibility of other new infectious agents (e.g. transmissible spongiform encephalopathies and severe acute respiratory syndrome). The introduction of the nucleic acid amplification can avoid the window period of these viral infections. Contamination from the laboratory environment can be achieved by routine screening for bacteria, fungi, yeast and mycoplasma by European pharmacopoeia tests. Fastidious micro-organisms, and an adventitious or endogenous virus, is a well-known fact that will also have to be considered for processes involving in vitro culture of stem cells. It is also a standard part of current good practice in stem cell banks to carry out routine environmental microbiological monitoring of the cleanrooms where the cell cultures and their products are prepared. The risk of viral contamination from products of animal origin, like bovine serum and mouse fibroblasts as a “feeder layer” for the development of

embryonic cell lines, should also be considered. Stem cell lines should be tested for prion particles and a virus of animal origin that assure an acceptable quality.

Introduction

The transplant of cells of human origin is a sector of medicine which shows increasing and exciting opportunities for the treatment of diseases, some of which until now have been incurable. To ensure the provision of safe and reliable cells and tissues for these applications, it is necessary to regulate the procurement, processing, testing, preservation, storage and distribution of all cells destined for application in the human body. These functions are entrusted to the tissue banks, although each one will have its differentiating nuances depending on the types of tissues handled and patient groups served. These establishments should be designed to meet the requirements for tissue banks as outlined in national recommendations (Department of Health 2001; American Association of Tissue Banks 2002) and under specific international standards (e.g. International Organization for Standardization 1999) and accredited by an appropriate competent authority (Directive 2004/23/CE). Although numerous tissue banks in Europe have been accredited by national inspection, there are specific requirements for banks of human stem cell lines, which have been addressed at the UK Stem Cell Bank (NIBSC), which was accredited by the Medical and Healthcare Products Regulatory Agency in 2004 (Pincock 2004; Mayor 2004) under the UK Code of Practice for Tissue Banks (2001) that is based on the UK Orange Guide (GPMD 2002), the UK Blood Transfusion Service “Red Book” (UKBTS/NIBSC 2002).

Stem cell banks must assure the quality, traceability and safety of these products, and these aims are particularly important in the avoidance of transmissible diseases. This should be achieved with close attention to the standardisation of processes and the implementation of quality control programmes and methods which reflect current best practices.

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Quality is important in all aspects of cell culture; good practice in the laboratory and the frequent monitoring of the cell lines is vital for any research centre that uses cell cultures, and would be mandatory if these cultures are intended for clinical use in cell therapy and regenerative medicine.

Tissue banks aim to screen all processed tissue for serious human pathogens and assure that no contaminants are introduced in the banking procedures, including storage. Thus, within the quality assurance programmes in these establishments, one of the fundamental pillars is the adequate preparation and introduction of a microbiological, environmental and biosafety quality assurance programme. Obviously, serious human pathogens present a risk to other processed tissues and staff as well as the recipient patients. Furthermore, any microbiological contamination of donor material introduced during the manufacturing process can potentially multiply during the processing stage and present a serious hazard to recipients even if it is not an overt pathogen. Each cell culture process provides ideal conditions for the growth of many organisms, including viruses. The use of antibiotics will not necessarily protect against microbial growth and may only mask the presence of certain organisms by inhibiting, but not eliminating, them and leaving a masked contaminant that may well infect any future recipient of a stem cell line. The four key areas in this microbiological control are:

- (1) Donors of biological material, including pre-embryos: appropriate screening should be carried out to avoid the transmission of infectious agents.
- (2) “Feeder” cells, cell line products, and other biological reagents and products used in cell culture procedures are further potential sources of contamination, and very strict controls should be established on their sourcing and quality control.
- (3) Environmental control, working conditions and laboratory plans for processing and storage areas.
- (4) Safety testing regimes for stem cell banks.

This review intends to give an overview of the methodology that should be used in this type of establishment and the microbiological controls that the cell lines should be submitted to in order to assure their quality and avoid the transmission of infections.

Control of donors of cells for derivation of stem cell lines

The main points to consider are fully informed consent, procedures and technical criteria to assess the eligibility of the donor, including the laboratory tests required and the criteria for the acceptance of cells.

With the exception of the case of couples donating reproductive cells for direct use (i.e. excluding storage), the donation of human cells should be assessed and approved by one person or persons (clinical advisor/s) who have appropriate experience with the use of tissues for clinical use. They should also have completed a specific training

programme to familiarise them with banking procedures (Department of Health 2001). In addition to the diagnostic tests or assessments for the presence of the risk of known transmissible diseases and other pathologies or harmful agents, extensive study of medical records and lifestyle questionnaires should be carried out.

All human cells (including reproductive cells) have the potential to transmit infectious diseases. In the past, donors have been screened for human immunodeficiency virus (HIV) and for hepatitis viruses, mainly B (HBV) and C (HCV) (US Food and Drugs Administration 1999; Department of Health 2000). These tests have been carried out using currently available protocols that are based on the detection of donor antibody response to viral infection. Research has shown that the detection of antibodies exclusively runs the risk of samples for tests being taken during an antibody-negative window period of these infections (i.e. absence of reactive antibodies), where an individual has been exposed to viral infection and indeed can be viraemic (Hitzler and Runkel 2001). HCV and HIV are potentially the agents of most concern in this category, where individuals have been shown to be negative for the presence of reactive antibodies but, however, are viraemic for a number of months before seroconversion (Schreiber et al. 1996; Stramer et al. 2004). This finding has led to the introduction of nucleic acid amplification techniques, such as polymerase chain reaction (PCR), in which the presence of a virus can be observed by means of the amplification of sequences in the viral genome. Although the prevalence rates of HBV, HCV, HIV and human T cell lymphotropic virus (HTLV) are lower among tissue donors than in the general population (Dodd et al. 2000), the estimated probability of undetected viremia at the time of tissue donation is higher among tissue donors than among first-time blood donors (Janssen et al. 1998; Dodd et al. 2002). The addition of nucleic acid test (NAT) methods to the screening of tissue donors, and to the testing of derived cell lines, should reduce the risk of these infections among recipients of stem cell lines (Zou et al. 2004).

Current regulations require that donor screening includes tests for a range of viruses causing serious human diseases and that are known to be transmitted by blood and tissues (Tables 1 and 2). These lists have been expanded for donors providing leucocyte-rich cells or tissues to include HTLV-I and HTLV-II, HCV and cytomegalovirus (CMV), which are considered “cell-associated viruses”. Additional viruses, such as hepatitis A and E, are also included in this associated-risk group and may need to be considered. It should be noted that national guidelines may vary. In Spain, the determination of hepatitis B surface antigen (HB_sAg), HIV-1 and HIV-2 antibody, HCV antibody, CMV antibody and toxoplasma antibody for heart transplants is mandatory. Also, the HTLV-I/HTLV-II antibodies determination would be made only when risk factors were identified for the donor (Consensus Document 1999). Although this list might be expanded even further in light of developing knowledge and technology, it is inevitable that a balance will be drawn between the

Table 1 Requirements for microbiological testing of all donors (Department of Health 2000)

Infection	Test
HIV-1 and HIV-2	HIV-1 and HIV-2 antibody
Hepatitis B	HB _s Ag ^{a,b}
Hepatitis C	HCV antibody
Syphilis ^c	Treponemal-specific antibody

^aRoutine anti-HBc (antibody to hepatitis B core antigen) testing is not advocated except for liver donation (including multi-organ donations involving the liver). For organ transplants other than the liver, where the anti-HBc status is known, donations positive for anti-HBc may be used as long as Hb_sAg is negative (see Table 2)

^bIn viral screening tests for Hb_sAg, undertaken on cadaveric blood samples, high rates of non-specific reactivity are recognized. Where confirmatory tests (antigen neutralisation on the initial test plus a second enzyme-linked immunoassay (EIA) test of equal or greater sensitivity) clearly indicate the absence of infection, material derived from donors, whose blood samples are repeat reactive, in Hb_sAg screening tests may be used

^cTesting transplant donors for syphilis has been maintained due to recent UK outbreaks. Reactive tests with treponemal specific antibodies require confirmatory testing

associated risk of infection and the resources and time required to perform an ever-increasing list of virus tests.

Currently, donor screening for non-viral agents may include syphilis (UK MSBT 2000), and it is inevitable that there will be a requirement to test for transmissible spongiform encephalopathies (TSEs), including Creutzfeldt–Jakob Disease (CJD), as sensitive and clinically validated assays become available (US Food and Drugs Administration 1999; European Medicines Evaluation Agency 1999). The Food and Drugs Administration (FDA) is proposing to require that donors of reproductive cells and tissue be tested for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (which are known to have been transmitted through artificial insemination) and screened for other sexually transmitted and genitourinary diseases that could contaminate reproductive cells and tissue during recovery

Table 2 Additional microbiological tests for specific indications (Department of Health 2000)

Infection	Indication	Test
CMV	For solid organ and allogenic bone marrow donors	CMV antibody ^a
Hepatitis B	Liver donors	Anti-Hbc ^b
Toxoplasma	Heart, liver and bone marrow donors (for patients not receiving cotrimoxazole prophylaxis after transplant)	Toxoplasma antibody

^aWhere possible, two assays should be used and a consensus achieved

^bIt is highly desirable to have anti-HBc results for liver donation (including multi-organ donations including the liver). However, the test currently has a high false-positive rate

and then be transmitted to the recipient of those cells or tissues (Table 3).

It is known that some viruses, such as herpesviruses [herpes simplex virus (HSV), Epstein–Barr virus, human herpesvirus (HHV-6, HHV-7 and HHV-8), human Polyomaviruses (JC and BK viruses), parvovirus B19 and transfusion transmitted virus (TTV)], remain latent and detectable in humans from early childhood and are potential contaminants of cells from normal, healthy individuals (Takeuchi et al. 1996; Cassinotti et al. 1997; Garbuglia et al. 2003; Fanci et al. 2004; Arnold et al. 2005). Most of these viruses could be screened for by PCR tests on the biological products; however, the work involved in establishing a clinically validated test cannot be underestimated and should include the establishment and use of appropriate national or international reference materials (Saldanha 2001). As these viruses are so ubiquitous, there may be no clinical impact of their presence in transplanted tissues and cells for the majority of patients, and in certain cases, such as parvovirus B19, contamination of blood products up to a maximum limit (currently 10⁵ genome equivalents per dose for B19) is acceptable for use in humans. However, in some instances, these agents can prove to be of concern. In heart transplant patients, the transfer of an organ from a CMV-positive donor to a recipient who has never been exposed to the virus can prove to be fatal, owing to the high degree of immunosuppression that is required for these patients to avoid rejection. Accordingly, all infectious agents should be considered as potential pathogens, and a risk balance for the use of contaminated products established, which should include consideration of the recipient's prior exposure and competence to fight infection.

Some microbial agents have marked variation in their geographical distribution, producing infectious epidemics in different areas. Recently, in the US, there has been a substantial increase in the incidence of disease caused by the West Nile virus (Centers for Disease Control and Prevention 2004). Most of these cases appear to have been

Table 3 Examples of serious diseases that may be transmitted in transplanted tissues and currently available screening tests (US Food and Drugs Administration 1999)

Infections	Diagnostic tests
HIV type 1	Anti-HIV-1; NAT
HIV type 2	Anti-HIV-2; NAT
HBV	HB _s Ag; anti-core HBc; NAT
HCV	Anti-HCV; NAT
<i>Treponema pallidum</i>	TPHA
HTLV-I and HTLV-II	Anti-HTLV-I and anti-HTLV-II
CMV	Ig G anti-CMV
Epstein-Barr virus	NAT
Transmissible spongiform encephalopathies	Western blot; NAT
<i>Neisseria gonorrhoeae</i> ^a	Bacterial culture
<i>Chlamydia trachomatis</i> ^a	Ig G anti- <i>Chlamydia</i>

NAT Nucleic acid test

^aFor donors of reproductive cells and tissue

asymptomatic, and many individuals will not know they are infected. Therefore, there is a potential risk that the cells and tissues donated can be infected by this type of virus. These viruses can multiply in the majority of human cells, and so, the virus can compromise biological products. It is noteworthy that this virus is only one of a group of arthropod-borne viruses that can infect humans, so awareness amongst tissue coordinators of new outbreaks of such disease or new emerging diseases is important in the safety of human transplantation and the use of tissue products.

The very recent outbreak of severe acute respiratory syndrome (SARS) virus in humans in South East Asia clearly demonstrated the potential for the appearance of new serious pathogens for which we have no means of screening donors of tissue. Obviously, any new entity that arises should be considered as a contamination risk factor, and donor records (e.g. frequent international traveler, vacation in high-risk areas) may be used as a first line of defence against contaminated donations. Furthermore, specific tests may be required, and these may be developed for surveillance initiatives, as in the case of SARS, for which detection methods are being developed for the causative coronavirus agent (Juang et al. 2004).

The risks of viral contamination from the donor may also be influenced by the cell types predominant in the tissue of origin which may predispose to certain types of contamination. This approach may be used to identify viruses more likely to be present; for example, lung tissue may carry the respiratory pathogens adenovirus, influenza virus and respiratory syncytial virus etc. Such approaches have been proposed for the risk assessment of cell lines (Dobhoff-Dier et al. 1999; Frommer et al. 1993) and might be applied to stem cell lines.

It is clear that before using human cell products, they should remain under quarantine and stored in a separate “in process” liquid nitrogen storage vessel until it is determined whether these products are acceptable based on the results of quality control and the screening tests performed by accredited laboratories for the relevant contaminants (Table 3).

Microbiological control of stem cell lines

The culture of cell lines *in vitro* involves numerous “open processes” (exposure of cultures to laboratory air) and intimate contact with a variety of reagents of animal origin. The origin of the cell lines can have an important effect on the quality, since the cell lines recently imported to the laboratory constitute the greatest source of contamination depending on their culture history and past exposure to micro-organisms. Thus, providers of cell lines should be able to provide details of passage history and appropriate testing (Stacey and Phillips 1999; Stacey et al. 2000). Once the cell lines have been obtained from a reliable source, it is important at the earliest stage to establish a master bank and apply appropriate tests to rule out microbiological contaminants and to confirm the authenticity of the culture.

On this matter, numerous articles (Nelson-Rees et al. 1981; MacLeod et al. 1999; Stacey et al. 2000) have identified and commented on the problem of cross contamination with different cell lines in a significant percentage of cell cultures from many laboratories, thus highlighting the importance of authentication.

Bacteria, fungi and yeast contamination

Bacterial contamination is usually evident to the naked eye by a sudden increase in turbidity and colour change of the culture medium as the result of a change in pH. The cell culture may survive for a short time, but the cells die eventually. Daily observation of cultures will ensure early detection of contamination and enable appropriate action to be taken as soon as the first signs of contamination become apparent to avoid contamination of other cultures. Moreover, specific tests for the detection of bacteria, yeast and fungi should be used as part of a routine and regular quality control screening procedure. To detect low levels of infection, samples from the cell cultures and their products may be inoculated in either liquid (e.g. tryptone soya broth and fluid thioglycollate medium) or onto solid (e.g. tripticase–soya agar, blood agar, Sabouraud’s dextrose agar and malt extract agar) growth media. Standard protocols for such testing are given as pharmacopoeial standards (European Pharmacopeia 2002; European Pharmacopeia 2004a,b). These inoculated media may be incubated at different temperatures, reflecting conditions for human pathogen culture (e.g. 35°C) and environmental organisms with lower growth temperature optima (e.g. 25°C) for 14 days in microbiological culture incubators depending on the specific testing standard used. Media and quality control equipment should be tested in parallel using challenge reference strains of potential contaminants. These procedures should be carried out in the microbiology laboratory isolated from the cell culture laboratory.

The sterility test is capable of detecting a large number of bacterial and fungal species. Membrane filtration of the product, with either an open or a closed system, is the preferred sterility test methodology (European Pharmacopeia 2002). The filter should be pre-wetted, particularly when small volumes and antibiotics are tested. If the product cannot be filtered, then direct inoculation, immersion, *in situ* incubation or combination methods, as appropriate, are acceptable. Validation studies should demonstrate that all media are capable of supporting the growth of a wide range of micro-organisms. Test containers should be inspected at intervals during the incubation period for the presence of turbidity (liquid media) or characteristic colonies (solid media), and these observations should be recorded.

The identification of micro-organisms is confirmed using confirmatory tests. Microbial identification systems are either manual (conventional procedures) or automated. Manual methods offer the advantage of using the analytical skills of the technologists for reading and interpreting the tests, whereas automated systems offer a hands-off ap-

proach, allowing more technologist time for other duties. For all systems, the backbone of accuracy is the strength and utility of the database (O'Hara et al. 2003).

Mycoplasma contamination

Mycoplasmas are smaller than bacteria (0.3 μm in diameter) and can be observed as filamentous or coccal forms. There are several species which are known to occur in 98% of the laboratory infections of cell cultures: *Mycoplasma hyorhinitis*, *Mycoplasma arginini*, *Mycoplasma orale*, *Mycoplasma fermentans*, *Mycoplasma salivarium*, *Mycoplasma hominis* and *Acholeplasma laidawii* (McGarrity 1982).

The effects of mycoplasma infection are more insidious than those produced by other bacterias, fungi and yeast. They may remain undetected by microscopic observation but can cause a reduction of growth rate, morphological changes, chromosomal aberrations, induction or suppression of cytokine expression, a change in membrane composition and alterations in the amino acid and nucleic acid metabolism (McGarrity et al. 1992).

Although mycoplasma contamination of primary cultures and continuous cell lines has been known for several decades, it still represents a significant problem in cell culture. This might be due to the inability of workers to detect these contaminants by microscopic observation. Mycoplasma contamination will also fail to be detected during routine sterility testing for other common bacterial, fungi or yeast infections due to their fastidious growth requirements, including the need for cholesterol. The surveys of cell culture laboratories and cell banks substantiate that on average, 15–35% of all cell cultures may be contaminated with mycoplasma (Drexler and Uphoff 2002; Uphoff and Drexler 2005). These data probably represent the highest expected incidence and should not be extrapolated to all laboratories where adequate screening and elimination of contaminated cultures is implemented. The infections mainly spread from one culture to another, transmitted by aerosols or by poor cell culture practice. Therefore, good laboratory practice and frequent monitoring of the cell lines is mandatory for every laboratory engaged in research using cell cultures (Hartung et al. 2002; Microbiological Control Methods 2003). A variety of tests for mycoplasma detection are available, and it is usually recommended to use at least two techniques for testing cell banks to ensure optimum sensitivity and specificity.

Mycoplasma detection by culture

Detection of mycoplasma by culture is the reference method of detection and has a theoretical level of detection of 1 colony-forming unit (CFU). However, there are some strains of mycoplasmas that are non-cultivable (e.g. strains of *M. hyorhinitis*). The colonies are observed on agar plates, with some species exhibiting a characteristic “fried egg”

appearance. The culture is carried out in specific solid (e.g. pleuropneumonia-like organisms (PPLO) agar plates and pig serum agar plates) and liquid (e.g. liquid PPLO medium and pig serum agar broths) media for 4 weeks in an aerobic and anaerobic atmosphere, including internal negative controls and the use of reference strains. A European pharmacopoeia test is published for this technique (European Pharmacopoeia 2004b). The culture media include thallos acetate to inhibit bacteria, but all isolates should be confirmed, and this can be achieved with PCR (see below).

Detection of Mycoplasma using indirect DNA staining (Hoechst 33258)

Hoechst 33258 is a stain that binds specifically to any DNA, and by using a UV epifluorescence microscope, the fluorescent nuclei and an extranuclear fluorescence revealing small cocci or filaments that correspond to the mycoplasma DNA are observed. Once again European pharmacopoeia methods are published (European Pharmacopoeia 2004b).

The method of indirect DNA staining of culture supernatants provides results within 24 h which compares favourably with 4 weeks for detection by culture. However, in this method, the sensitivity is very much reduced (10^6 CFU/ml). This may be improved by co-culturing the test cell line, in the presence of an indicator cell line such as Vero, which provides a surface to adhere to and grow the mycoplasma. This enrichment step results in a sensitivity of 10^4 CFU/ml.

Mycoplasma detection using PCR

The PCR technology has also been adapted to detect mycoplasmas in cell cultures. This method combines a number of advantages that renders it a very competitive and reliable method. PCR is a very sensitive, specific and rapid procedure which detects defined DNA sequences by amplification of the target DNA sequences and by visualization of the fragment on an ethidium bromide stained gel or by simultaneous amplification–detection in real time PCR.

Another important advantage of the PCR technology is its simplicity because, once established, it can be used routinely by cell culturists in any cell culture or molecular biology laboratory without any training in classical mycoplasma detection (Toji et al. 1998).

On the other hand, this method can be affected by inhibitors, which might be introduced from cell culture media, due to rapid preparation procedures used (e.g. supernatant denatured by boiling or a raw cell lysate treated with proteinase K instead of purified DNA). The bacterial contamination of the PCR reagents can themselves lead to an amplification that is obviously not backed up in cell culture contaminations (Razin 1994), especially in nested PCR methods.

Before testing for mycoplasma, the use of antibiotics in cell culture should be minimized, and the cells should be cultured without antibiotics for several passages, or at least 2 weeks, to allow the mycoplasmas to grow to detectable amounts.

The regions of the genome which have been conserved can be used as targets for the primers for the detection of all the species. One of the most often-chosen conserved regions for the amplification is the 16S rRNA coding region (Stacey 2000). The use of this region is advantageous, as many copies (e.g. 10^4 copies) will be available which could be reverse transcribed into DNA. This high copy number increases the sensitivity of the PCR method (Barry et al. 1990).

Detection of mycoplasma using the GenProbe mycoplasma tissue culture non-isotopic detection system

This method uses the principle of nucleic acid hybridization and of ribosomal RNA (rRNA) detection (Weisburg et al. 1989). It is possible to detect positive samples in 75 min. The mycoplasma tissue culture (MTC) non-isotopic (NI) kit uses an all-bacterial probe that detects all species of *Mycoplasma* and *Acholeplasma* which commonly infect the cell cultures.

This kit uses only a single-stranded DNA probe with a DNA chemiluminescent label which is complementary to the rRNA of the target micro-organism. Likewise, stable DNA-RNA hybrids that are measured in a GenProbe luminometer (Nelson et al. 1995) are formed. A positive result is a luminometer reading equal to or greater than the cut-off value expressed in the relative light units.

This method has been tested for a wide range (22) of species of *Mycoplasma*, *Acholeplasma*, *Spiroplasma* and *Ureaplasma*, for which this method is adequate with respect to its detection. However, the sensitivity of detection is low (e.g. $>10^5$).

Contamination by fastidious micro-organisms

In addition to mycoplasma there are numerous types of micro-organisms that may contaminate and persist in cell cultures and go undetected without specific isolation methods. This is the case with some members of the *Mycobacterium* genus and other bacteria with demanding nutritional requirements. To isolate such micro-organisms a very different culture and conditions will be required for their detection, and it will not be possible to cover all types of potential contamination. Nevertheless, it is wise to remain aware of the potential for rare contamination of this type and to be prepared to attempt to isolate such organisms should unusual observations arise that could be explained by such contaminants.

Use of anti-microbials in cell cultures

For routine clean cell culture it is best to restrict the use of antibiotics for primary cell cultures. In the event of cell cultures becoming contaminated with bacteria, yeast, fungi or mycoplasmas, the best course of action is to discard the culture, check cell culture reagents for contamination which have been used, as well as disinfect all the microbiological safety cabinets and work surfaces, and to resuscitate new ampoules of cells from the frozen stock. In the case of contamination with spore-forming micro-organisms, it is advisable to fumigate the work areas if there are facilities to do so.

However, in the case of irreplaceable stocks, the treatment with anti-microbials may be necessary to eliminate the contamination, although it must be acknowledged that such attempts will rarely be successful. In this case, the cells should be cultivated in the presence of antibiotics for 10–14 days. Each passage should be performed at the highest dilution of cells at which growth is possible. If the contaminant is still detectable after this period of time, it is unlikely that the anti-microbial used will prove successful, and another antibiotic should be tried if possible.

Checks for contamination should be carried out after treatment (e.g. about 5–7 days after treatment in the case of bacteria and fungi, and approximately 20–30 days after treatment for mycoplasma-contaminated lines).

The antibiotic chosen to eliminate a contaminant depends on the sensitivity of the micro-organism in question. The performance of tests to determine the species of organism and the minimum inhibitory concentration (MIC) of the antibiotic will be useful to promote eradication. It is important to remember that cells may be susceptible to the antibiotic toxic effects, and thus, selection of MIC levels avoids the use of excessive concentrations (Doyle and Griffiths 1998).

Contamination by virus in cell culture

Some lines or cell cultures may contain an endogenous virus or may be contaminated with exogenous viruses, and may secrete viral particles or express viral antigens on their surface. The primary sources of potential viral contamination come from infected animal tissue used to prepare biological reagents and media and contamination during laboratory manipulation. In addition, infected laboratory workers may cause contamination of stem cell lines during culture manipulation.

Cell banks should be submitted to a panel of tests to detect serious pathogens, other endogenous viruses and adventitious viruses. This panel of tests usually includes electronic microscopy, reverse transcriptase (RT) detection (as a general test for retroviruses), in vivo and in vitro tests for infectious virus, tests to induce an antibody response in animals (e.g. mouse antibody production tests and rat

antibody production tests) and other specific tests to find human, bovine, porcine or rodent viruses depending on the origin of the biological products used in the cultures (International Conference on Harmonisation 1997). Some of these tests may also need to be applied to culture reagents of animal origin, and any testing performed by the manufacturer should be carefully evaluated before accepting the reagents for use. Tests on cells should normally be performed on samples taken directly from the established cell banks, and one possible model for testing is presented in Tables 4 and 5.

There are two tests that are included in the screening of other biological products that are used to screen for a wide range of viruses: the in vitro cell culture assay and the study in animals (European Medicines Evaluation Agency 1997). These are long standing techniques that rely on the ability of many viruses to cause cytopathic changes in cell culture or clinical changes in animals. These tests can take a number of weeks, and therefore, can prove difficult to implement in the scheduling for release testing of time-critical products. The only useful alternative solution today is to expand the number of PCR and RT-PCR tests to include all viruses that we know pose a risk to the product.

The PCR test is a simple, in vitro and chemical reaction that permits the synthesis of essentially limitless quantities of a targeted nucleic acid sequence. This is accomplished through the action of a DNA polymerase that, under the proper conditions, can copy a DNA strand (Nolte and Caliendo 2003).

The RT-PCR is a technique in which cDNA is first produced from RNA targets by reverse transcription, and

Table 4 Tests of cell bank contamination recommended in the characterization of cell lines (modified from Dellepiane et al. 2000)

Test	Master bank	Work bank	Cells at the passage limit for clinical use
Electronic microscopy	+	–	+
Reverse transcriptase	+	–	+
Other tests for human pathogenic viruses	^a	–	^a
Tests for viruses from other species of animal	^a	–	–
In vitro cell inoculation	+	–	+
In vivo animal inoculation	+	–	+
Rodent antibody Production tests ^b	+	–	–
Sterility	+	+	+
Mycoplasma	+	+	+
Identify test (DNA isoenzyme)	+	+	–
Karyology	+	+	+

^aSpecific tests according to regulatory guides

^bAccording to risk assessment or and regulatory guidance

Table 5 Tests of detection of adventitious virus recommended in the characterization of cell lines (Dellepiane et al. 2000)

Test	Master bank	Work bank	Cells in the passage limit
In vitro assays	+	– ^a	+
In vivo assays	+	– ^a	+
Antibody production	+	–	–

^aFor the first work bank, this test should be carried out with cells in the limit of age cellular

then the cDNA is amplified by PCR (Nolte and Caliendo 2003).

It is not possible to assert the absolute absence of viral contaminants, and reports on viral testing must be qualified to address the influence of a number of factors, including:

- sample size and likelihood of detecting low-level virus,
- impossibility of covering all potential viral contaminants,
- sensitivity of tests used and inhibitory effects of other materials in sample and
- specificity of tests and potential to miss closely related species/strains.

Potential adventitious viruses that can be introduced during production

These viruses can be introduced into the final product by several routes, including the use of contaminated biological reagents, such as animal serum components, the use of a virus for the induction of expression of specific genes encoding a desired protein, the use of a contaminated reagent as a monoclonal antibody and the use of a contaminated excipient (e.g. cryoprotectant) during the formulation, the use of murine or human feeders for embryonic stem cell culture and the contamination during cell and medium handling.

A very common supplement for cell culture is bovine serum. New guidelines describing the screening of bovine serum before its use in the manufacturing of a human biological product have recently been introduced (European Medicines Evaluation Agency 2003). These guidelines are designed for the quality control and safety of bovine serum used during the manufacturing of human biological medicinal products. The serum should not contain any detectable bacteria, fungi or mycoplasmas. The virus testing list should include known bovine pathogens, including bovine viral diarrhoea virus (BVDV), bovine polyomavirus, bovine parvovirus, bovine adenovirus, blue tongue virus, rabies virus, bovine respiratory syncytial virus and reovirus. The serum should be sourced from countries where these viruses are not present or have low incidence.

The contamination of cell lines with BVDV may cause slight changes in growth rate, but since this virus is non-cytopathic, macroscopic and microscopic changes in the culture will not be detected by immunoperoxidase. It is

preferable to use a semi-quantitative RT-PCR with an internal control which will allow quantification of the number of detected BVDV RNA molecules in each sample for the detection of BVDV. This should allow discrimination between background signals and real BVDV contamination (Rijsewijk et al. 2002).

Embryonic stem cell lines derived with mouse feeders and those derived with human feeders can transmit infectious micro-organisms to the recipient. There is evidence that certain mouse viruses, like Hantaan virus, reovirus type 3, Sendai virus, lactic dehydrogenase virus and lymphocytic choriomeningitis virus are capable of infecting humans or primates (European Medicines Evaluation Agency 1997). Furthermore, other viruses, like ectromelia virus, Minute virus of mice, mouse adenovirus, mouse cytomegalovirus, mouse rotavirus, pneumonia virus of mice, Toolan virus and Kilham rat virus are capable of replying in vitro in cells of human or primate origin, although infective capacity has not been shown in them to date (European Medicines Evaluation Agency 1997). In the event of using feeder cells of human origin, there are numerous viruses and other infectious agents that are susceptible to being transmitted to the recipient (see [Control of donors of cells for derivation of stem cell lines](#)).

In the future, the technologies based on the use of hybridization chips, using microarrays of immobilized oligonucleotides, can provide a rapid and useful methodology of the identification of contaminants (Laassri et al. 2003).

The microchip contained several-bases-long oligonucleotide probes specific to each virus species to ensure the redundancy and robustness of the assay. A region, approximately 1,100 bases long, was amplified from samples of viral DNA and fluorescently labelled with Cy5-modified deoxynucleotide triphosphates (dNTPs), and single-stranded DNA was prepared by strand separation. Hybridization was carried out under plastic coverslips, resulting in a fluorescent pattern that was quantified using a confocal laser scanner (Laassri et al. 2003). However, such techniques will need to be carefully validated for sensitivity and specificity before being applied in the safety testing of human tissue products and stem cell lines for therapy.

The European regulatory agencies (European Medicines Evaluation Agency 2003) have identified the need for a risk assessment for TSEs in all products derived from ruminants. Human beings can be exposed to secondary infections of TSE/CJD using medical procedures or by administering biological products derived from humans, including blood (Llewelyn et al. 2004; Peden et al. 2004). As a result of this, preventive measures should be implemented with respect to blood products, cell or tissue grafts and the decontamination of surgical instruments. The diagnosis in these cases can be carried out by means of the detection of resistant prionic protein (PrP^{res}) by different methods: chemiluminescence, immunoassays in sandwich, Western blot, enzyme-linked immunosorbent assay (ELISA) sandwich and PCR. Although the specificity of these diagnostic methods is nearly 100% (Ironsides 1996; Lee et al. 2000; Wadsworth et al. 2001), the sensitivity is inadequate to assure the value of a negative

result. An alternative to these methods would consist of the use of transgenic mice; these would be vaccinated with tissues or fluids that have unknown infectivity of the cell lines which have used bovine serum to be obtained.

Environmental microbiological control

It is good practice in any stem cell bank, whether for research or therapy, to carry out routine environmental monitoring of the laboratory where the cell cultures and their products are prepared. Class II microbiological safety cabinets should be reviewed every 6 months to assure that they carry out their function to protect the product and operator effectively. There are a variety of methods for detecting and monitoring environmental air quality and contaminants, including the use of devices, settle plates and contact plates or swabs (EC Guide to Good Manufacturing Practice 2003; European Pharmacopoeia 2004a,b). Settle plates are most effective when used to study different points throughout the laboratory and identify foci of contamination risk. These plates should be open for approximately 4 h; after this time, they should be covered and incubated at 37°C for 48 h (Parenteral Society 2002). At the end of this period the plates should be examined to rule out the presence of micro-organism growth. To obtain environmental monitoring data that relates to ISO air quality standards (e.g. International Organization for Standardization 1999) an air sampling system can be used to which a contact plate can be attached. This system samples large air volumes and provides greater reliability with respect to the microbial charge at each point in the laboratory space.

Other environmental monitoring activities in the laboratory should include testing samples of the work surfaces, walls, equipment and instruments using convex culture plates and swabs. In the case of the latter, once used for sampling in the laboratory, they should be introduced in liquid mediums, like thioglycollate broth or tryptone soya agar. Broth cultures are then subcultured onto agar plates.

Certain levels of contamination are acceptable, but limits should be defined in terms of a “warning level” and “action level”, and these depend on how critical the work area is and the levels of cleanliness that can be reached under normal operative conditions.

Conclusion

Cell banks for stem cell lines arise from the necessity to guarantee the existence of an appropriate source of such cell lines in a standardised way for the development of therapies through clinical trials. In addition to providing a reliable and reproducible source of cells, the advantages of a system of accredited cell banks includes the assurance of safe starting materials for use in clinical trials that have also been checked for authenticity, stability and performance.

Potential sources of microbial contamination include other cell lines, reagents, laboratory environment and breakdown in aseptic processing. The availability of good quality

cells and reagents from qualified sources is not only necessary to guarantee the quality of the production process and the final products (cell stocks and cell products). Routine screening regimes help in the early detection of contamination, since any type of manipulation is a potential source of contamination.

Validation of quality control and safety testing methods is vital to assure the quality of test data and also reduce the need to repeat tests. These procedures should be carried out and documented according to applicable national requirements and international standards where materials may be transferred across borders.

Although the most rigorous testing should be performed for serious human pathogens, other tests are performed to detect human and animal viruses that are not desirable in a stem cell line for use in human therapy. In some cases these agents may be of very low or undetermined pathogenicity and may not affect the decision to use the cells for therapy; however, in the use of stem cell lines, there is the possibility that such agents can multiply to unacceptable levels, and this must be monitored.

Safe practice in the laboratory is based on some fundamental norms that are included in the good microbiological practice for the safe manipulation of micro-organisms that may arise in tissues and cells. The operators should have basic knowledge of microbiology as well as knowledge of how to cultivate pathogens. Likewise, they should have basic knowledge of techniques of disinfection of work surfaces, equipment and the environment. They should be aware of the need to minimize the production of aerosols and control other potential mechanisms of the spread of contamination between tissues and workers.

To assure the quality of the cell lines requires authentication, characterisation and accurate description and provenance and to confirm the absence of contaminants. These aims are important for stem cell lines for both research and clinical use, and the appropriate documentation should be provided to recipients of the cell lines.

The products derived from stem cell lines for cell therapy and regenerative medicine can provide new solutions for serious disease and injury. There are still many problems to be solved before the final and routine application in humans will be realised. Sensitive diagnostic tests that provide complete biosafety for these products are necessary, and it will also be vital to apply improved detection technology and to maintain vigilance for new potential agents that affect the biosafety of stem cell lines.

References

- American Association of Tissue Banks (2002) Standards for tissue banking, 10th edn. American Association of Tissue Banks, McLean, VA
- Arnold DM, Neame PB, Meyer RM, Soamboonsrup P, Luinstra KE, O'hoski P, Garner J, Foley R (2005) Autologous peripheral blood progenitor cells are a potential source of parvovirus B19 infection. *Transfusion* 45:394–398
- Barry T, Powell R, Gannon F (1990) A general method to generate DNA probes for microorganisms. *Biotechnology* 8:233–236
- Cassinotti P, Burtonboy G, Fopp M, Siegl C (1997) Evidence for persistence of human parvovirus B19 DNA in bone marrow. *J Med Virol* 53:229–232
- Centers for Disease Control and Prevention (2004) West Nile virus screening of blood donations and transfusion associated transmission—United States, 2003. *MMWR Morb Mortal Wkly Rep* 53:281–284
- Consensus Document (1999) Selection criteria in the organ donors respect to transmission of infections. Grupo GESITRA (SEIMC), Spain
- Dellepiane N, Griffiths E, Milstien JB (2000) New challenges in assuring vaccine quality. *Bull World Health Organ* 78:155–162
- Department of Health (2000) Guidance on the microbiological safety of human organs, tissues and cells used in transplantation. Advisory committee on the Microbiological Safety of Blood and Tissues for Transplantation, MSBT. Department of Health, London, UK
- Department of Health (2001) A code of practice for tissue banks. Department of Health, London, UK
- Directive 2004/23/CE of the European parliament and the council of March 31st, relating to the establishment of quality and safety norms to donate, to obtain, to assess, to process, to preserve, to store and to distribute cells and human tissues
- Doblhoff-Dier O, Bachmayer H, Bennett A, Brunius G, Burki K, Cantley M, Collins C, Crooy P, Elmqvist A, Frontali-Botti C, Havenacer R, Haymerle H, Lelieveld H, Lex M, Mahler JL, Martinez L, Mosgaard C, Olsen L, Pazlarova J, Rudan F, Sarvas M, Stepankova H, Tzotzus G, Wagner K, Werner R (1999) Safe biotechnology 9: values in risk assessment for the environmental application of microorganisms. The safety in Biotechnology Working Party of the European Federation of Biotechnology. *Trends Biotechnol* 17:307–311
- Dodd RY, Stramer JL, Aberle-Grasse J, Notari E IV (2000) Risk of hepatitis and retroviral infections among blood donors and introduction of nucleic acid testing (NAT). *Dev Biol Stand* 102:19–27
- Dodd RY, Notari EP IV, Stramer SL (2002) Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion* 42:975–979
- Doyle A, Griffiths JB (1998) Elimination of contamination. In: *Cell and tissue culture: laboratory procedures in biotechnology*. Wiley, New York
- Drexler HG, Uphoff CC (2002) Mycoplasma contamination of cell cultures. Incidence, sources, effects, detection, elimination, prevention. *Cytotechnology* 39:75–90
- EC Guide to Good Manufacturing Practice (2003) Manufacture of sterile medicinal products, revision to annex 1. EC, Brussels
- European Medicines Evaluation Agency (1997) ICH Consensus guideline on quality of biotechnology products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin, publication no CPMP/ICH/295/95. European Medicines Evaluation Agency, Canary Wharf, London
- European Medicines Evaluation Agency (1999) EMEA workshop on application of pharmaceutical assays for markers of TSE: report to CPMP from the BIOT-WP (CPMP/BWP/257/99). EMEA, London
- European Medicines Evaluation Agency (2003) Note for guidance on the use of bovine serum in the manufacture on human biological medicinal products. EMEA, London
- European Pharmacopeia (2002) Pharmaceutical inspection convention. Recommendation on sterility testing, PIC/s
- European Pharmacopeia (2004a) European Pharmacopeia section 2.6.1 (Sterility). Maisonneuve SA, Sainte Ruffine
- European Pharmacopeia (2004b) European Pharmacopeia section 2.6.7 (Mycoplasma). Maisonneuve SA, Sainte Ruffine
- Fanci R, De Santis R, Zakrzewska K, Paci C, Azzi A (2004) Presence of TT virus DNA in bone marrow cells from hematologic patients. *New Microbiol* 27:113–117

- Frommer W, Archer L, Boon B, Brunius G, Collins CH, Crooy P, Doblhoff-Dier O, Donikian R, Economidis J, Frontali C (1993) Safe biotechnology (5). Recommendations for safe work with animal and human cell cultures concerning potential human pathogens. *Appl Microbiol Biotechnol* 39:141–147
- Garbuglia AR, Iezzi T, Capobianchi MR, Pignoloni P, Pulsoni A, Sourdis J, Pescarmona E, Vitolo D, Mandelli F (2003) Detection of TT virus in lymph node biopsies of B-cell lymphoma and Hodgkin's disease, and its association with EBV infection. *Int J Immunopathol Pharmacol* 16:109–118
- GPMD (2002) Rules and guidance for pharmaceutical manufacturers and distributors (EEC Orange Guide). The Stationery Office, London
- Hartung T, Balls M, Bardouille C, Blank O, Coecke S, Gstramthaler G, Lewis D (2002) Good cell culture practice. ECVAM Good Cell Culture Practice Task Force Report 1. *ATLA* 30:407–411
- Hitzler WE, Runkel S (2001) Screening of blood donations by hepatitis C virus polymerase chain reaction (HCV-PCR) improves safety of blood products by window period reduction. *Clin Lab* 47:219–222
- International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (1997) ICH harmonised tripartite guideline. Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. ICH, Geneva
- International Organization for Standardization (1999) ISO 14644. Cleanrooms and associated controlled environments. Part 1: Classification of air cleanliness. International Organization for Standardization
- Ironside JW (1996) Review: Creutzfeldt–Jakob disease. *Brain Pathol* 6:379–388
- Janssen RS, Satten GA, Stramer SL, Rawal BD, O'Brien TR, Weiblen BJ, Hecht FM, Jack N, Cleghorn FR, Kahn JO, Chesney MA, Busch MP (1998) New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA* 280:42–48 (Erratum, *JAMA* 1999; 281: 1893)
- Juang JL, Chen TC, Jiang SS, Hsiong CA, Chen WC, Cheng GW, Lin SM, Lin JH, Chiu SC, Lai YK (2004) Coupling multiplex RT-PCR to a gene chip assay for sensitive and semiquantitative detection of severe acute respiratory syndrome coronavirus. *Lab Invest* 84:1085–1091
- Laassri M, Chizhikov V, Mikheev M, Shchelkunov S, Chumakov K (2003) Detection and discrimination of orthopoxviruses using microarrays of immobilized oligonucleotides. *J Virol Methods* 112:67–78
- Lee DC, Stenland CJ, Hartwell RC, Ford EK, Cai K, Miller JL, Gilligan KJ, Rubenstein R, Fournel M, Petteway SR (2000) Monitoring plasma processing steps with a sensitive Western blot assay for the detection of the prion protein. *J Virol Methods* 84:77–89
- Llewellyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, MacKenzie J, Will RG (2004) Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. *Lancet* 363:417–421
- MacLeod RA, Dirks WG, Matsuo Y, Kaufmann M, Milch H, Drexler HG (1999) Widespread intraspecies cross-contamination of human tumor cell lines arising at source. *Int J Cancer* 83:555–563
- Mayor S (2004) First stem cell bank in the world is opened in UK. *BMJ* 328:1277
- McGarrity GJ (1982) Detection of mycoplasma infection of cell cultures. In: Maramorosch K (ed) *Advances in cell cultures*, vol. 2. Academic, New York, pp 99–131
- McGarrity GJ, Kotani H, Butler GH (1992) Mycoplasmas and tissue culture cells. In: Maniloff J, McElhaney RN, Fich LR, Baseman JB (eds) *Mycoplasmas—molecular biology and pathogenesis*. American Society for Microbiology, Washington, D.C., pp 445–454
- Microbiological Control Methods (2003) In the European Pharmacopeia: present and future. European Directorate for the Quality of Medicines Meeting, Copenhagen, pp 5–7 and 29–31
- Nelson NC, Reynolds MA, Arnold LJ Jr (1995) Detection of acridinium esters by chemiluminescence. In: Kricka LJ (ed) *Non isotopic probing, plotting and sequencing*. Academic, San Diego, CA, pp 391–428
- Nelson-Rees WA, Daniels DW, Flandermeyer RR (1981) Cross-contamination of cells in culture. *Science* 212:446–452
- Nolte FS, Caliendo AM (2003) Molecular detection and identification of microorganisms. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Tenover FC, Tenover FC (eds) *Manual of clinical microbiology*. ASM, Washington, D.C., pp 234–256
- O'Hara CM, Weinstein MP, Miller JM (2003) Manual and automated systems for detection and identification of microorganisms. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Tenover FC (eds) *Manual of clinical microbiology*. ASM, Washington, D.C., pp 185–207
- Parenteral Society (2002) Parenteral Society Technical Monograph No 2. Environmental contamination control practice. Parenteral Society, London
- Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 264:527–529
- Pincock S (2004) UK opens embryonic stem cell bank. *Lancet* 363:1778
- Razin S (1994) DNA probes and PCR in diagnosis of mycoplasma infections. *Mol Cell Probes* 8:497–511
- Rijssewijk F, Maris-Veldhuis MA, Oei HL, Claassen I (2002) Semi-quantitative detection of BVDV RNA in vaccine samples using RT-PCR with internal control RNA molecules. *ID-lelystad/CIDC*
- Saldanha J (2001) Validation and standardisation of nucleic acid amplification technology (NAT) assays for the detection of viral contamination of blood and blood products. *J Clin Virol* 20:7–13
- Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ (1996) The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. *N Engl J Med* 334:1685–1690
- Stacey GN (2000) Detection of mycoplasma by DNA amplification. In: Doyle A, Griffiths JB (eds) *Cell and tissue culture for medical research*, chapter 2. Wiley, New York, pp 58–61
- Stacey GN, Phillips P (1999) Quality assurance for cell substrates. *Dev Biol Stand* 98:141–151
- Stacey GN, Masters JRW, Hay RJ, Drexler HG, MacLeod RA, Freshney RI (2000) Cell contamination leads to inaccurate data: we must take action now. *Nature* 403:356
- Stramer SL, Glynn SA, Kleinman SH, Strong M, Caglioti S, Wright DJ, Dodd RY, Busch MP (2004) Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid amplification testing. *N Engl J Med* 351:760–768
- Takeuchi H, Kobayashi R, Hasegawa M, Hirai K (1996) Detection of latent infection by Epstein–Barr virus in peripheral blood cells of healthy individuals and in non-neoplastic tonsillar tissue from patients by reverse transcription-polymerase chain reaction. *J Virol Methods* 58:81–89
- Toji LH, Lenchitz TC, Kwiatkowski VA, Sarama JA, Mulivor RA (1998) Validation of routine mycoplasma testing by PCR. In *Vitro Cell Dev Biol*, Anim 34:356–358
- UK Code of Practice for Tissue Banks (2001) Providing tissues of human origin for therapeutic purposes, Department of Health, Crown
- UK MSBT (2000) Guidelines on the microbiological safety of human organs, tissues and cells used in transplantation. Advisory Committee on the Microbiological Safety of Blood and Tissues for Transplantation, MSBT
- UKBTS/NIBSC (2002) Guidelines for the blood transfusion services in the United Kingdom (Red Book), 6th edn. UKBTS/NIBSC, London
- Uphoff CC, Drexler HG (2005) Detection of mycoplasma contaminations. *Methods Mol Biol* 290:13–23

- US Food and Drugs Administration (1999) CFR (Code of Federal Regulations) section 1271 Subpart C-suitability determination for donors of human cellular and tissue based products, proposed rule 64 FR 189. US Food and Drugs Administration, Rockville, MD
- Wadsworth JD, Joiner S, Hill AF, Campbell TA, Desbruslais M, Luthert PJ, Collinge J (2001) Tissue distribution of protease resistant prion protein in variant Creutzfeldt–Jakob disease using a highly sensitive immunoblotting assay. *Lancet* 358:171–180
- Weisburg WG, Tully JG, Rose DL, Petzel P, Oyaizu H, Yang D, Mandelco L, Sechrest J, Lawrence TG, Van Etten J, Maniloff J, Woese CR (1989) A phylogenetic analysis of the mycoplasmas: basis of their classification. *J Bacteriol* 171:6455–6467
- Zou S, Dodd RY, Stramer SL, Strong M (2004) Probability of viremia with HBV, HCV, HIV and HTLV among tissue donors in the United States. *N Eng J Med* 351:751–759